



## Cancer Research Center Hotline

### Local Studies Address a Previously Hidden Sexually Transmitted Disease: Human Papillomavirus and Cervical Neoplasia

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Of the many triumphs in our fight against cancer, perhaps the best recognized has been the significant decrease in cervical cancer incidence worldwide through the use of the Papanicolaou (Pap) screening test. As with many other public health successes, routine cytological screening and treatment of dysplasia and carcinoma *in situ* were performed without complete knowledge of the cause(s) or natural history of cervical malignancy. Our understanding of cervical neoplasia and the carcinogenic process has come a long way since the introduction of the Pap smear, and we now know that the majority of cervical cancer is caused by a single infectious agent, the human papillomavirus (HPV).<sup>1</sup> What we don't know is why only certain women develop cervical dysplasia or cervical cancer when HPV infection appears relatively common; nor why Asian/Pacific Islanders in Hawaii generally have lower incidence rates of HPV-associated squamous cell carcinomas than whites.<sup>2</sup>

In 1991, prompted by the availability of a new DNA amplifying technique, polymerase chain reaction (PCR), we initiated a case-control study of risk factors for pre-malignant changes in cervical epithelium. The specific aims of this five-year investigation, funded by the National Cancer Institute, were to identify viral and non-viral risk factors for cervical dysplasia, with a special emphasis on the interaction of HPV and diet. Eligible subjects included non-hysterectomized women, from 18 to 84 years of age, who were residents of Honolulu. Women who had been pregnant or lactating within 6 months of enrollment, who had a diagnosis of cervical abnormalities within the past three years, or who were clinic referrals were considered ineligible for study. Eligible cases were identified through the cytology logs of the participating clinics and included all women with a cytological classification of atypical squamous cells of undetermined significance (ASCUS) or squamous intraepithelial lesion (SIL) according to the Bethesda system.<sup>3</sup> Potentially eligible women were contacted by letter, followed by a telephone call, for consent to participate in the study before their return to the clinic for a follow-up examination and cervical smear. As part of the study, a colposcopy was performed and cervical cells were collected for HPV testing. Interview information was obtained from 214 women with biopsy-confirmed SIL and 254 women with a cytological diagnosis of ASCUS. Blood was drawn from 147 of the SIL cases and 185 of the ASCUS cases who completed interviews.

Controls were women with negative cytological smears attending

the same clinics as the cases. Eligible women were selected from the admission logs of the participating clinics on a randomly selected day of the month. Potential controls were met at the clinics by one of the interviewers who explained the purpose of the study. An exfoliated cervical cell specimen was obtained at the time of the cervical cytological smear. Of the 271 women with negative cytological results who were interviewed, 191 further consented to have their blood drawn.

Participants were scheduled for a personal interview at their homes or other convenient location. A standardized questionnaire was used to elicit a detailed sexual and reproductive history, including age at first intercourse, number of sexual partners and methods of contraception, diet, a lifetime history of tobacco and alcohol use, and other demographic and lifestyle information. Fasting blood was used to determine plasma levels of a variety of nutrients. DNA was purified from peripheral blood leukocytes by SDS/proteinase K treatment and phenol/chloroform extraction for genetic analysis. Frozen cervical cell specimens were prepared for HPV-DNA detection by PCR amplification and dot blot hybridization of the amplicons.

Our findings suggest that high plasma concentrations of several antioxidants may reduce the risk of ASCUS<sup>4</sup> and cervical SIL.<sup>5</sup> We found an inverse dose-response of  $\alpha$ -cryptoxanthin, total tocopherol, and  $\alpha$ -tocopherol to the odds ratios for cervical SIL, after adjustment for HPV, tobacco smoking, and alcohol drinking. The risk of cervical dysplasia was reduced by about 70% among women in the highest compared with the lowest quartile of these micronutrients. We were also interested in the influence of dietary and plasma folate levels and related compounds on the risk of cervical dysplasia. Folate is essential to the synthesis of nucleotides, and low levels of folate have implications for cell replication, DNA excision and repair, and DNA hypomethylation. We found an inverse association of dietary,<sup>6</sup> but not plasma,<sup>7</sup> levels of folate, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>, on the risk of cervical dysplasia.

Other than diet, we have also explored several genetic hypotheses regarding the etiology of cervical dysplasia. Most recently, we examined the risk associated with a polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene.<sup>6</sup> A common variant of *MTHFR*, which catalyzes the synthesis of 5-methyltetrahydrofolate, results from a 677C6T (*ala6val*) substitution in the gene. This variant has been shown to have reduced enzymatic activity and is associated with mild hyperhomocysteinemia. We found a positive, monotonic trend ( $p = 0.02$ ) in the risk of cervical SIL associated with the number of variant *MTHFR* T alleles. Women with the heterozygous CT genotype had twice the risk of cervical SIL, and women with the homozygous TT genotype had almost three times the risk of SIL, compared to women who were homozygous for the wild type (CC) *MTHFR* genotype.

Tobacco smoking has been associated with the risk of cervical malignancy and SIL,<sup>3</sup> and nicotine, cotinine, and tobacco-specific nitrosamines have been detected in the cervical mucus of smokers.<sup>8</sup> Although the significance of these observations is unclear, DNA adducts of bulky aromatic compounds have been found with increased frequency in the cervical epithelium of smokers compared to nonsmokers,<sup>9</sup> providing biochemical evidence that smoking may be a cause of cervical cancer. Based on the significant positive association of tobacco smoking and cervical dysplasia in this investigation, we explored the hypothesis that genetic polymorphisms in

the cytochrome P450 1A1 (*CYP1A1*) and glutathione S-transferase classes mu and theta (*GSTM1* and *GSTT1*) genes promote dysplastic change by moderating the activation and detoxification of polycyclic hydrocarbons and other compounds that influence oxidative stress and DNA adduct formation. Women who were homozygous, but not heterozygous, for the *CYP1A1* *MspI* variant allele were at significantly increased risk of cervical SIL compared to women who were homozygous for the wild-type allele.<sup>10</sup> Subjects with the *GSTM1* null genotype were also at (borderline) elevated risk of cervical SIL compared to women with the gene present. No difference in the risk of cervical disease was associated with the *GSTT1* variant genotype. The combination of the *CYP1A1* homozygous variant and the *GSTM1* null genotypes increased the odds ratio for cervical SIL to 5.1 (95% confidence interval: 1.3-20.8). It is possible that both the *CYP1A1* *MspI* and *GSTM1* polymorphisms are susceptibility factors for early, premalignant changes in the cervical epithelium.

The recognition that HPV infection may be a necessary cause of cervical cancer has had a major impact on the epidemiological study of this disease. Of critical scientific and public health importance is whether the presently established risk factors for cervical dysplasia, such as tobacco smoking, are HPV cofactors that modify the progression of HPV infection to cancer or are simply correlated with viral infection. In collaboration with several local health care providers, we are presently engaged in another National Cancer Institute-funded project to establish a multiethnic cohort of 1,150 HPV-positive women for long-term follow-up to identify factors that influence the persistence or resolution of HPV infection of the cervix. The project aims are 1) to study the association of the dietary intake of fruits and vegetables and the plasma levels of carotenoids, tocopherols, and vitamin C with HPV persistence, and 2) to examine the role of HPV type, viral quantity, and multiple HPV infections in HPV persistence. The identification of these factors may provide insight into the natural history of HPV infection and may improve the ability to characterize women who are at greatest risk for developing HPV-associated neoplasia. A unique feature of this investigation is the development of a laboratory capability at the Cancer Research Center to perform HPV-testing. This is the only such laboratory in Hawaii and has been used increasingly by local physicians.

At present, we have enrolled over 1,000 local women of whom 210 were HPV-positive on the first or subsequent visits. We are asking for your help in enrolling additional volunteers. Participants in the study will be monitored through quarterly screening during a 3 year follow-up period. At each visit, a Pap smear is obtained, along with a blood specimen and collection of cervical cells for HPV testing. All participants will be reimbursed for their time and travel. Women at least 18 years old, not currently pregnant, and who have no prior history of hysterectomy are eligible for the study. Call Dr. Brenda Hernandez at (808)586-2987 for more information about participation in this study.

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